Journal of Surgical Oncology 1998;69:99–104

RAPID COMMUNICATION

CXCR-4, a Chemokine Receptor, Is Overexpressed in and Required for Proliferation of Glioblastoma Tumor Cells

ANIL SEHGAL, PhD,[1]* CASSIE KEENER, BS,[1] ALTON L. BOYNTON, PhD,[2] JAMI WARRICK, BS,[1] AND GERALD P. MURPHY, MD, DSc[1]

[1]*Pacific Northwest Cancer Foundation, Northwest Hospital, Seattle, Washington*
[2]*Molecular Medicine, Northwest Hospital, Seattle, Washington*

Background and Objectives: Using the technique of differential hybridization of Atlas™ Human cDNA expression arrays, we previously reported the isolation of a G protein coupled receptor, CXCR-4, which is overexpressed in glioblastoma multiforme tumor tissue (GMTT) compared to normal brain tissue (NBT).

Methods: Using gene specific reverse transcriptase-polymerase chain reaction (RT-PCR) and in situ hybridization, we studied its expression in a variety of brain and breast tumor tissue samples. To demonstrate the requirement of CXCR-4 in glioblastoma cell proliferation an antisense construct was overexpressed. Glioblastoma cells were also treated with antibodies against CXCR-4 and its ligand, SDFβ-1.

Results: Expression analysis indicated that CXCR-4 is overexpressed in 57% of the primary glioblastoma tissues and in 88% of the glioblastoma cell lines analyzed. Overexpression of CXCR-4 in glioblastoma cell lines enhanced their soft agar colony-forming capability. Expression of antisense CXCR-4 in glioblastoma cell lines caused neurite outgrowth and cellular differentiation. Treatment of glioblastoma cell lines with CXCR-4 and SDFβ-1 specific antibodies caused inhibition of glioblastoma cell proliferation.

Conclusions: On the basis of these results, we conclude that CXCR-4 gene is required for the proliferation of human glioblastoma tumors.

J. Surg. Oncol. 1998;69:99–104.

KEY WORDS: **G protein; chemokine; glioma; brain tumors**

INTRODUCTION

Chemokine receptors play an important role in the chemotaxis of T cells and phagocytic cells to areas of inflammation and also in their homing process during development [1]. CXCR-4 was first identified as a cDNA that was amplified using degenerate primers made against leukocyte chemotactic factor receptors [N-formyl peptides C5a and interleukin-8 (IL-8)] and was termed HM89 [2]. Ligand binding analysis showed that HM89 was not an N-formyl peptide receptor and sequence analysis clearly demonstrated that it is a member of the G protein coupled receptor family. Cytogenetic analysis indicates that HM89 is localized to human chromosome 2q2 [3]. HM89 was later recloned using a rabbit IL-8 receptor cDNA upon screening a human monocyte library and was named LESTR (leukocyte derived seven transmembrane domain receptor) [4]. HM89 was again

Grant sponsor: Pacific Northwest Cancer Foundation, Northwest Hospital, Seattle, WA.

*Correspondence to: Anil Sehgal, PhD, Pacific Northwest Cancer Foundation, 120 Northgate Plaza, Room 230, Seattle, WA 98125. Fax No.: (206) 368-3009. E-mail: asehgal@nwbio.org

Accepted 17 August 1998

cloned and identified as a cofactor for human immunodeficiency virus type 1 (HIV-1) fusion and entry into CD4+ cells [5]. This cofactor was identical to the previously cloned HM89, and because of its role as a fusion protein between the HIV-1 virus and CD4+ cells it was designated as ''fusin.'' Fusin in conjunction with CD4 was sufficient to allow HIV-1 entry into non-permissive murine 3T3 cells [6]. Sequence analysis has shown that HM89 and fusin are the same gene. Because of its chemoattraction properties, these genes are now termed CXC-chemokine receptor-4 (CXCR-4). Recently, it was shown that the CD4 independent infection by HIV-1 was mediated by the CXCR-4 receptor [7]. Interaction and cytopathic effects caused by entry of HIV-2 into CD4− cells were inhibited by a monoclonal antibody to the CXCR-4 protein [7]. The role of CXCR-4 in HIV infection was further strengthened when its introduction into human and non-human CD4-cells allowed HIV-2 infection [6]. The ligand for CXCR-4 was recently cloned and termed PBSF/SDF-1 (pre-B-cell growth stimulating factor/stromal cell derived factor-1) [8,9]. Transgenic mice that lack PBSF/SDF-1 died prenatally and their B cells and myeloid progenitors were severely reduced in numbers [10]. This result suggests that PBSF/SDFβ-1 is responsible for B-cell lymphopoiesis and bone marrow myelopoiesis.

Our investigations center on the identification of genes that are overexpressed in human glioblastoma tumors. Serendipitously, using the array technology, we identified that CXCR-4 gene is overexpressed in glioblastoma multiforme tumor tissue (GMTT) compared to normal brain tissue (NBT). Expression analysis indicated that CXCR-4 is overexpressed in a number of glioblastoma tumor tissues and cell lines. The role of the CXCR-4 gene in human glioblastomas has not been reported previously. In this study, we provide evidence that CXCR-4, in the presence of its ligand SDFβ-1, is required for the proliferation of glioblastoma tumor cells. In conclusion, this study demonstrates a previously unreported novel role of CXCR-4 in the genesis of human glioblastoma tumors.

MATERIALS AND METHODS

Human Tissues and Cell Lines

Primary human tumor tissue samples of brain and non-brain tumors were procured from the tissue bank maintained by Pacific Northwest Cancer Foundation, Northwest Hospital. Brain tumor cell lines were purchased from American Type Culture Collection (ATCC; Rockville, MD). Fetal normal human astrocytes (FNHA) were purchased from Clonetics (San Diego, CA). All of the cell lines were cultured under the conditions recommended by ATCC or Clonetics.

Differential Hybridization of Atlas™ Human cDNA Expression Arrays

Two Atlas™ Human cDNA expression array membranes were purchased from Clontech (Palo Alto, CA). Each membrane contained the cDNAs from 588 known genes and 9 housekeeping genes [11]. Total RNA was isolated from human GMTT and NBT and was then treated with DNaseI and first-strand cDNA synthesis was carried out in the presence of $dATP^{32}$. Equal amounts of cDNA from GMTT and NBT were then hybridized to two identical Atlas™ Human cDNA expression arrays in separate bags. The expression arrays were washed and then exposed to X-ray film. Details of this method have been described previously [11].

Gene Specific Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

To confirm the differential expression of genes identified on the expression array, we used the technique of gene specific RT-PCR as described previously [11]. PCR amplification of CXCR-4 was carried out using primers (5′ ctctccaaaggaaagcgaggtggacat3′ and 5′ agactgtacactgtaggtgctgaaatca3′). Hybridization of RT-PCR Southern blots was done by multiprime labeling of CXCR-4 primer (5′ atctgtttccactgagtctgatcttcaagttttcacccagctaacaca3′). D1-2 is a housekeeping mitochondrial cytochrome C oxidase subunit 1 gene that has been used as an internal control for RT-PCR [11,12]. PCR amplification of D1-2 was carried out using primers (5′cggagcaatatgaaatgatct3′ and 5′gcaaatacagctcctattg3′).

Cloning and Subcloning of Full Length CXCR-4 Gene

Full length CXCR-4 cDNA was isolated upon screening a human fetal brain library (Strategene, La Jolla, CA). CXCR-4 gene was then cloned into pCMV-neo vector after PCR amplification of its coding region with specific primers containing SacII and SpeI (underlined sites), (5′agatagatccgcggaccatggaggggatcagtatata3′ and 5′tagatacaactagtgtgttagctggagtgaaaacttga3′). The pCMV-neo vector then was digested with SacII and SpeI and ligated with CXCR-4 PCR product predigested with SacII and SpeI. To clone the CXCR-4 in the antisense direction, CXCR-4 specific primers (5′agatagatccgcgggtgttagctggagtgaaaacttga3′ and 5′tagatacaactagtaccatggaggggatcagtatata3′) were used for carrying out the PCR, digested with SacII and SpeI, and then cloned into the predigested pCMV-neo vector in the sense and antisense directions. Orientation of CXCR-4 gene was confirmed by sequencing.

Soft Agar Colony Forming Assay

Soft agar colony forming assay was done as described previously [13]. Glioblastoma GB1690 cells that were

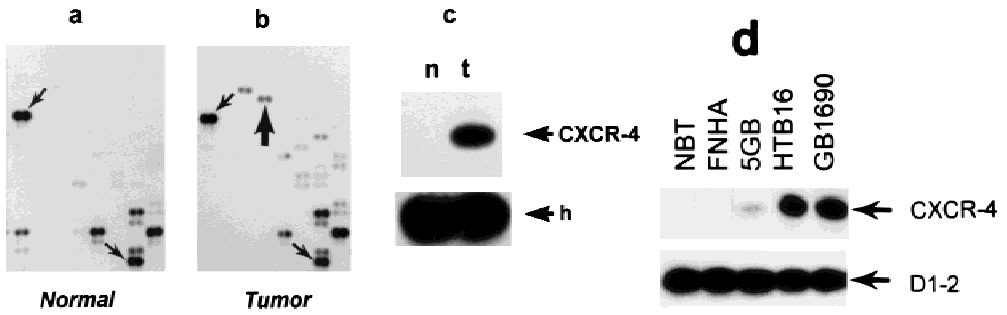

Fig. 1. Identification of the CXCR-4 gene using the technique of differential hybridization of Atlas™ Human cDNA expression arrays. **a:** Expression array hybridized with cDNA from NBT. **b:** Expression array hybridized with cDNA from GMTT. The CXCR-4 gene is indicated by a heavy arrow in b. Two other genes that were expressed at similar levels in both normal and tumor tissue are indicated by small arrows. **c:** CXCR-4 expression in normal (n) and tumor (t) tissue using the gene specific RT-PCR technique. D1-2, a housekeeping gene, is indicated by the letter h. **d:** Expression of CXCR-4 in glioblastoma cell lines.

transfected with pCMV-neo vector alone and with pCMV-neo CXCR-4 in the sense direction were trypsinized. Approximately 1×10^6 cells were mixed with 0.26% agar. Cells were then plated on top of a layer of 0.65% agar in 60 mm Petri dishes and incubated at 37°C for 2–4 weeks. Cells were fed with serum containing media every 10 days. Colonies containing more than 20 cells were counted under the inverted light microscope.

Antibody Neutralization Assay

A CXCR-4 polyclonal (rabbit anti-human) antibody was made against a synthetic peptide (MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANFNK) corresponding to the first 38 amino acids of CXCR-4 protein in rabbit (Genmed Synthesis, Inc., South San Francisco, CA). Approximately 1×10^3 cells (NIH3T3 and glioblastoma) were plated in 60 mm Petri dishes. Forty-eight hours after plating, a 1:50 final dilution of CXCR-4 polyclonal antibody or preimmune serum (from the same rabbit) was added to the culture media. Cells were harvested after 192 hr and counted on a cell counter. The values are the means of three separate determinations. Antibody against SDFβ-1 was purchased from R&D Systems (Minneapolis, MN). Twenty-four hours after plating 1×10^3 cells, anti-SDFβ-1 antibody was added to a final concentration of 30 μg/ml. Cells were harvested every 48 hr and counted on a cell counter.

RESULTS

Identification of CXCR-4 Gene by Differential Hybridization of Expression Arrays

An approach to the identification of altered gene expresion in GMTT compared to NBT is the technique of different hybridization of Atlas Human cDNA expression arrays [11]. This technique was previously used to isolate differentially expressed genes in breast carcinomas [14]. Using this technique, we found that the CXCR-4 gene is overexpressed in GMTT compared to NBT (Fig. 1a,b). To confirm the differential expression of CXCR-4 in GMTT, the technique of gene specific semiquantitative RT-PCR was used [11]. Figure 1c shows that CXCR-4 was expressed at high levels in GMTT with little or no expression in NBT. RT-PCR analysis indicated that CXCR-4 was overexpressed in three glioblastoma cell lines (5GB, HTB-16, and GB1690) (Fig. 1d).

Expression Analysis of CXCR-4 Gene in Human Tumor Tissue Samples

To further strengthen this finding, a total of 19 different glioblastoma tumors were tested for CXCR-4 expression using either the technique of in situ hybridization (8 tumors) or RT-PCR (11 tumors). Eleven of the nineteen glioblastoma tumors showed overexpression of CXCR-4 (Table I), while normal tissue from brain did not express CXCR-4. CXCR-4 was also overexpressed in the single surgical specimen we tested of glioma and meningioma tumors (data not shown). We also found that CXCR-4 was overexpressed in several brain tumor cell lines. Three different human glioblastoma brain tumor cell lines, GB1690, 5GB, and HTB-16, all overexpressed CXCR-4 between 10- and 200-fold (Table I, Fig. 1d), while primary cultures of normal fetal human astrocytes did not express CXCR-4. Other human brain tumor cell lines including neuroblastoma, neuroectoderm, medulloblastoma, and astrocytoma grade III tumors all overexpressed CXCR-4. Not unexpectedly, the CXCR-4 ligand SDF-1 was expressed in all of the primary glioblastoma, meningioma, malignant glioma, and neuroblastoma tumor surgical specimens that also overexpressed the CXCR-4 receptor (data not shown).

Because CXCR-4 was overexpressed in breast adenocarcinoma cell lines and because normal human breast tissue and breast adenocarcinoma tissue were readily available from the Pacific Northwest Cancer Foundation Tissue Bank, we determined CXCR-4 expression in these tissues. CXCR-4 was overexpressed at least 6-fold in 3 of 5 breast adenocarcinoma surgical specimens compared to

TABLE I. Overexpression of CXCR-4 in Primary Tumor and Normal Tissues and Cell Lines*

Tumor type	Source	No. of tissues with CXCR-4 overexpression/total no. of tissues analyzed
Brain tumors	Primary tissue	11/19
	Cell lines	8/9
Normal brain	Primary tissue	0/10
Breast tumors	Primary tissue	3/5
	Cell lines	3/7
Normal breast	Primary tissue	0/6

*Gene specific RT-PCR and Southern blotting were carried out using CXCR-4 and D1-2 specific primers for studying expression in brain and breast primary tissue and cell lines. Quantitation of Southern blots was performed using the ImageQuaNT^{TM} volume quantitation program from the Molecular Dynamics Phosphor Imager. Experiments were carried out twice and a 2-fold or greater CXCR-4 expression in tumor compared to NBT was considered overexpression. Eight of the 19 human brain glioblastoma surgical tissues were analyzed using the technique of in situ hybridization.

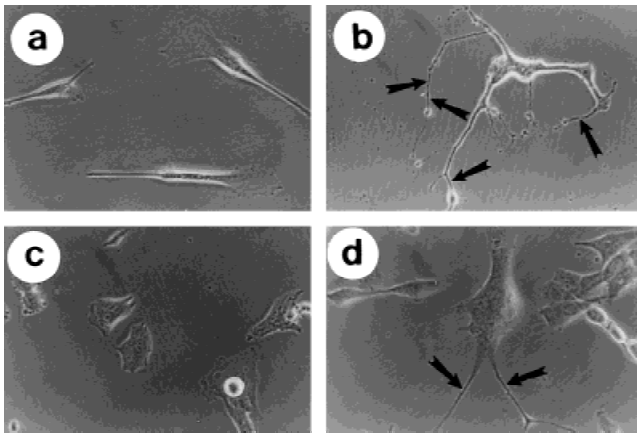

Fig. 2. The effect of CXCR-4 expression in the sense and antisense directions on the 5GB and GB1690 glioblastoma cell lines. **a,b:** Glioblastoma 5GB cells transfected with pCMV-neo vector and pCMV-neo CA (CXCR-4 antisense), respectively. **c,d:** Glioblastoma GB1690 cells transfected with pCMV-neo vector and pCMV-neo CA (CXCR-4 antisense), respectively. Neurite outgrowths are indicated by arrows.

normal breast surgical tissues (obtained from reduction mammoplasty; Table I).

Effect of Antisense CXCR-4 Expression on Glioblastoma Cell Morphology and Soft Agar Colony Formation

To demonstrate the role of CXCR-4 in glioblastoma proliferation, it was cloned into the pCMV-neo vector in the sense and antisense directions and transfected into the 5GB and GB1690 glioblastoma tumor cell lines. Within the first 2 weeks both cell lines transfected with the antisense CXCR-4 demonstrated neurite outgrowth and cellular differentiation, while cells transfected with sense CXCR-4 or vector only showed no changes in cell morphology (Fig. 2a,b). Similar change in cell morphology was observed when CXCR-4 antisense was overexpressed in glioblastoma GB1690 cells (Fig. 2c,d). Neurite outgrowth is a typical characteristic of differentiating cells such as glioblastoma cells in response to sodium butyrate [15] or neuroblastoma cells in response to retinoic acid (RA) and forskolin treatment [16]. By 3 weeks after transfection and selection in G418, all cells transfected with the antisense CXCR-4 had undergone differentiation followed by cell death.

Effect of CXCR-4 Overexpression on Soft Agar Colony Formation in Glioblastoma Cells

A role for CXCR-4 in cell proliferation was further investigated by transfecting and overexpressing a full length CXCR-4 cDNA into three different glioblastoma tumor cell lines. Enhanced proliferative activity was found in all three cell lines and in fetal normal human astrocytes (data not shown). We next investigated if overexpression of CXCR-4 alters the ability of the GB1690 cell line to form colonies in soft agar. As shown in Figure 3, overexpression of CXCR-4 caused 87.5% increase in the number of colonies formed. Colonies formed by CXCR-4 overexpressing GB1690 cells are much larger than cells transfected with vector alone.

Effect of CXCR-4 and SDFβ-1 Antibody Treatment on the Proliferation of Glioblastoma Cells

To confirm the requirement of CXCR-4 and SDFβ-1 in glioblastoma cell proliferation, we studied the effect of CXCR-4 and SDFβ-1 antibodies on glioblastoma cell lines (Fig. 4). Specific CXCR-4 polyclonal antibodies or preimmune serum were added to cultures 24 hr after plating. In two cell lines (GB1690 and 5GB), cell proliferation was inhibited by 50% while in the third line, HTB-16, cell proliferation was inhibited by 90%. NIH3T3 cells treated with CXCR-4 antibody showed no effect on cell proliferation (Fig. 4a). We next studied the effect of SDFβ-1 monoclonal antibody on the proliferation of glioblastoma cell lines. As shown in Figure 4b, SDFβ-1 antibody caused approximately 90% inhibition of cell proliferation of three glioblastoma cell lines (5GB, HTB-16, and GB1690). Conversely, treatment of NIH3T3 cells with SDFβ-1 antibody did not effect cell proliferation. These results clearly demonstrate that CXCR-4 and its ligand SDF-1 are required for glioblastoma cell proliferation.

DISCUSSION

The role of CXCR-4 receptor overexpression in neoplastic disease of the brain and breast is not presently understood. The fact that the CXCR-4 receptor and its ligand, SDFβ-1, are expressed by brain and breast tumors but not normal tissue suggests an autocrine and/or paracrine mechanism regulating proliferative behavior.

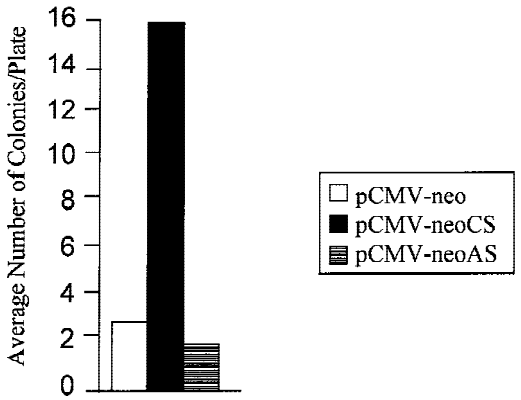

Fig. 3. The effect of CXCR-4 overexpression on colony formation in soft agar of glioblastoma cell line GB1690.

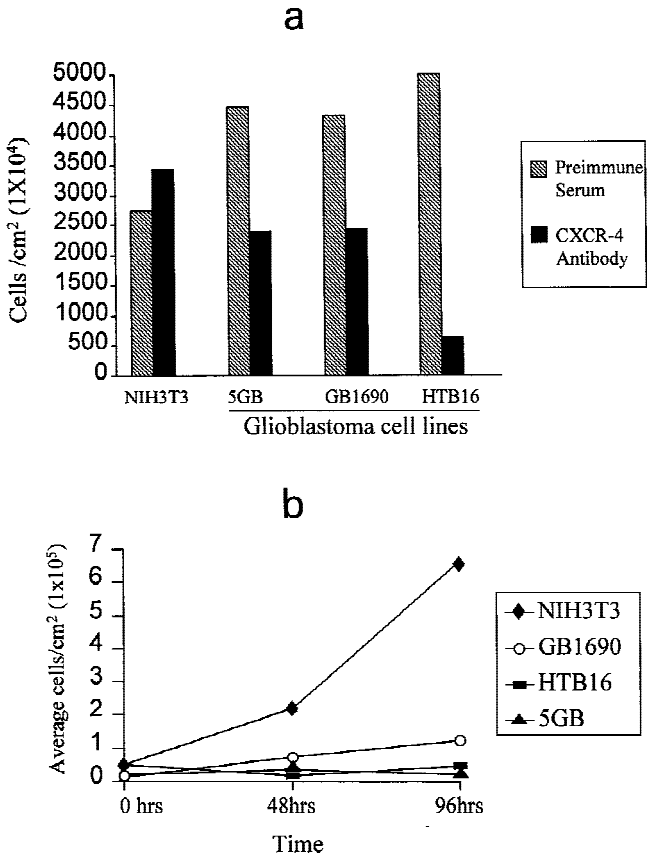

Fig. 4. The effect of CXCR-4 polyclonal antibody and SDFβ-1 monoclonal antibody treatment on the proliferation of glioblastoma tumor cell lines. Panel **a** shows the effect of CXCR-4 antibody treatment on glioblastoma cell lines. Panel **b** shows the effect of SDFβ-1 antibody treatment on glioblastoma cell lines.

In a recent study, it was demonstrated that SDFβ-1 binding to its receptor, CXCR-4, induces hydrolysis of phosphatidylinositol 4,5-bisphosphate yielding inositol 1,4,5-trisphosphate, which releases Ca^{2+} from intracellular stores, and diacylglycerol, which activates protein kinase C [17]. Furthermore, an influx of extracellular Ca^{2+} follows which is known to be required for sustained proliferative activity. Thus, an autocrine/paracrine loop involving SDFβ-1 secretion followed by binding to its receptor, CXCR-4, is sufficient to explain how the CXCR-4 chemokine receptor is responsible for maintaining the aberrant proliferative behavior of neoplastic cells. Additionally, the cytoplasmic tail of CXCR-4 receptor contains a number of phosphorylation sites, some of which are believed to be involved in receptor desensitization. These include sites for protein kinase C, calmodulin-dependent protein kinases, and tyrosine protein kinases [17]. Receptor desensitization can also occur in a phosphorylation independent manner, apparently through the phosphorylation and down-regulation of PLCβ3 [17].

Several other members of the G protein coupled receptor (GPR) family are known to be involved in the process of cell transformation including (1) MAS (neuronal angiotensin sensitive receptor); (2) serotonin 1c receptor; (3) muscarinic acetylcholine receptors m1, m3, and m5; and (4) α_{1B}-adrenergic receptor [18–21]. In these GPRs, both ligand dependent and independent mechanisms have been observed which are responsible for cell transformation. Northern blot analysis of CXCR-4 in leukemic cells demonstrated the absence of any structural aberrations (data not shown). The possibility of specific mutations in one or more of these sites is not known but could contribute to a constitutively active receptor that is stimulated by the autocrine/paracrine production of the SDFβ-1 ligand.

The CXCR-4 chemokine receptor is a molecule with pleiotropic functions. In addition to its defined role in the entry of HIV into CD4+ cells and chemotaxis, CXCR-4 has other functions such as in brain embryonic development and during vascular homeostasis [22,23]. The overexpression of the CXCR-4 receptor in glioblastoma and breast adenocarcinomas is a remarkable finding in light of its role in HIV infection. Our unique observations such as CXCR-4 antibody and antisense mediated inhibition of glioblastoma cell proliferation strongly suggest a novel role for CXCR-4 in the maintenance of the neoplastic phenotype of glioblastoma cells. Moreover, the concomitant overexpression of the CXCR-4 ligand SDFβ-1 by these cancers suggests an autocrine/paracrine role in the genesis of aberrant proliferative behavior of glioblastoma tumors.

ACKNOWLEDGMENTS

We thank Ronald F. Young, MD, Sandra S. Vermeulen, MD, Kenneth S. Yonemura, MD, Erik P. Kohler, MD, Hector C. Aldape, MD, and Charles R. Simrell, MD, for providing human brain tissue samples. We also thank Sherianne Ricks, BS, for her technical contribution to this study.

REFERENCES

1. Baggiolini M, Dewald B, Moser B: Human chemokines: An update. Annu Rev Immunol 1997;15:675–705.

2. Nomura H, Nielsen B, Matsushima K: Molecular cloning of cDNAs encoding a LD78 receptor and putative leukocyte chemotactic peptide receptors. Int Immunol 1993;5:1239–1249.
3. Federsppiel B, Melhado IG, Duncan AMV, et al.: Molecular cloning of the cDNA and chromosomal localization of the gene for a putative seven-transmembrane segment (7-TMS) receptor isolated from human spleen. Genomics 1993;16:707–712.
4. Loetscher M, Geiser T, O'Reilly T, et al.: Cloning of a human seven-transmembrane domain receptor, LESTR, that is highly expressed in leukocytes. J Biol Chem 1994;269:232–237.
5. Doranz BJ, Berson JF, Rucker J, et al.: Chemokine receptors as fusion cofactors for human immunodeficiency virus type 1 (HIV-1). Immunol Res 1997;16:15–28.
6. Feng Y, Broder CC, Kennedy PE, et al.: HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 1996;272:872–877.
7. Endres MJ, Clapham PR, Marsh M, et al.: CD$-independent infection by HIV-2 is mediated by fusin/CXCR4. Cell 1996;87:745–756.
8. Bleul CC, Fuhlbrigge RC, Casasnovas JM, et al.: A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). J Exp Med 1996;184:1101–1109.
9. Bleul CC, Farzan M, Choe H, et al.: The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature 1996;382:829–833.
10. Nagasawa T, Hirota S, Tachibana K, et al.: Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996;382:635–638.
11. Sehgal A, Boynton AL, Young RF, et al.: Application of the differential hybridization of Atlas™ Human expression arrays technique in the identification of differentially expressed genes in human glioblastoma multiforme tumor tissue. J Surg Oncol 1998; 67:234–241.
12. Sehgal A, Keener C, Boynton AL, et al.: Isolation and characterization of a novel gene from human glioblastoma multiforme tumor tissue. Int J Cancer 1997;71:565–572.
13. Huang RP, Liu C, Fan Y, et al.; Erg-1 negatively regulates human tumor growth via the DNA binding domain. Cancer Res 1995;55: 5054–5062.
14. DeRisi J, Penland L, Brown PO, et al.: Use of a cDNA microarray to analyze gene expression patterns in human cancer. Nat Genet 1996;14:457–460.
15. Engelhard HH, Duncan HA, Canto MD: Molecular characterization of glioblastoma cell differentiation. Neurosurgery 1997;41: 886–897.
16. Moore TB, Koeffler HP, Yamashiro JM, et al.: Vitamin D_3 analogs inhibit growth and induce differentiation in LA-N-5 human neuroblastoma cells. Clin Exp Metastasis 1996;14:239–245.
17. Harihabu B, Richardson RM, Fisher I, et al.: Regulation of human chemokine receptor CXCR4. J Biol Chem 1997;272:28726–28731.
18. Jackson TR, Blair LAC, Marshall J, et al.: The mas oncogene encodes an angiotensin receptor. Nature 1988;355:437–440.
19. Julius D, Livelli TJ, Jessell TM, et al.: Ectopic expression of the serotonin 1c receptor and the triggering of malignant transformation. Science 1989;244:1057–1062.
20. Gutkind JS, Novotny EA, Brann MR, et al.: Muscarinic acetylcholine receptor subtypes as agonist dependent oncogenes. Proc Natl Acad Sci USA 1991;88:4703–4707.
21. Hen R, Axel R, Obici S: Activation of the β_2-adrenergic receptor promotes growth and differentiation in thyroid cells. Proc Natl Acad Sci USA 1989;86:4785–4788.
22. Hesselgesser J, Miller MH, DelVecchio V, et al.: CD-4 independent association between HIV-1 gp120 and CXCR4: Functional chemokine receptors are expressed in human neurons. Curr Biol 1997;7:112–121.
23. Volin MV, Joseph L, Shockley MS, et al.: Chemokine receptor CXCR4 expression in endothelium. Biochem Biophys Res Commun 1998;242:46–53.